

Preliminary Observations on the Fine Structure of Species of *Micromonospora* (Actinomycetales)

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THE GENUS *Micromonospora* was first described by Ørskov in 1923 as a member of the Actinomycetales characterized by unicellular mycelium and the production of single, terminal conidia. The genus was little known for some time (Jensen, 1930), but subsequently the frequent occurrence of micromonosporae in soil (Jensen, 1932), lake bottoms (Erikson, 1941; Potter and Baker, 1956) and lake water (Potter and Baker, 1956), became well recognized. In spite of this wide acquaintance with the genus, the general morphology and cell structure is still not well understood and descriptions are sometimes at variance. The chemical composition of the actinomycete wall, including micromonosporae, (Erikson, 1947; Avery and Blank, 1951; Yamaguchi, 1965) is better documented than is the general morphology of the cell structure. Ultrastructure studies are few to date, and provide little detail (Agre, 1962; Leudemann and Brodsky, 1964). Both of these studies were concerned primarily with spores. A paper by Arai, Koyama, Kuroda, and Honda (1964) is more definitive for both mycelium and spores. It also includes some electron micrographs.

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The small size of the mycelium and spores in the micromonosporae has made study with the light microscope difficult and, in part, accounts for the lack of critical morphological and cytological information. Waksman (1961) characterized the substrate or vegetative hyphae as straight or curved, branching, and without cross-walls. The lack of cross-walls has been accepted widely (Jensen, 1930; Erikson, 1941; Krasil'nikov and Agre, 1965), although in 1964 Arai et al. illustrated septa.

There is increasing recognition of the economic importance of the micromonosporae as sources of antibiotics (Leudemann and Brodsky, 1964; Weinstein, Leudemann, Oden, and Wagsman, 1965) and as etiologic agents of disease in man (Castellani, De Brito, and Pinto, 1959). In view of this and of the discrepancies among reports on their structure, there is need for a consistent comparative study of the general cell structure and the process of spore production in the group. Consequently, studies at the ultrastructural level were undertaken for three species: *Micromonospora fusca* Jensen (isolated from Flathead Lake water, Potter, No. M-1012); *M. purpurea* Leudemann and Brodsky (NRRL No. 2953); and *M. sp.*, isolated from a foot lesion at the University of Miami. All cultures came from the collection of Dr. Louise F. Potter, Biology Department, Elmira College, Elmira, New York.

FIG. 1. *Micromonospora fusca*, showing the electron-dense cytoplasm and fibrillar nucleoid. The two roundish masses probably represent prespores. $\times 32,000$.

FIG. 2. Spore of *Micromonospora fusca*, showing the sculptured spore wall. $\times 26,000$.

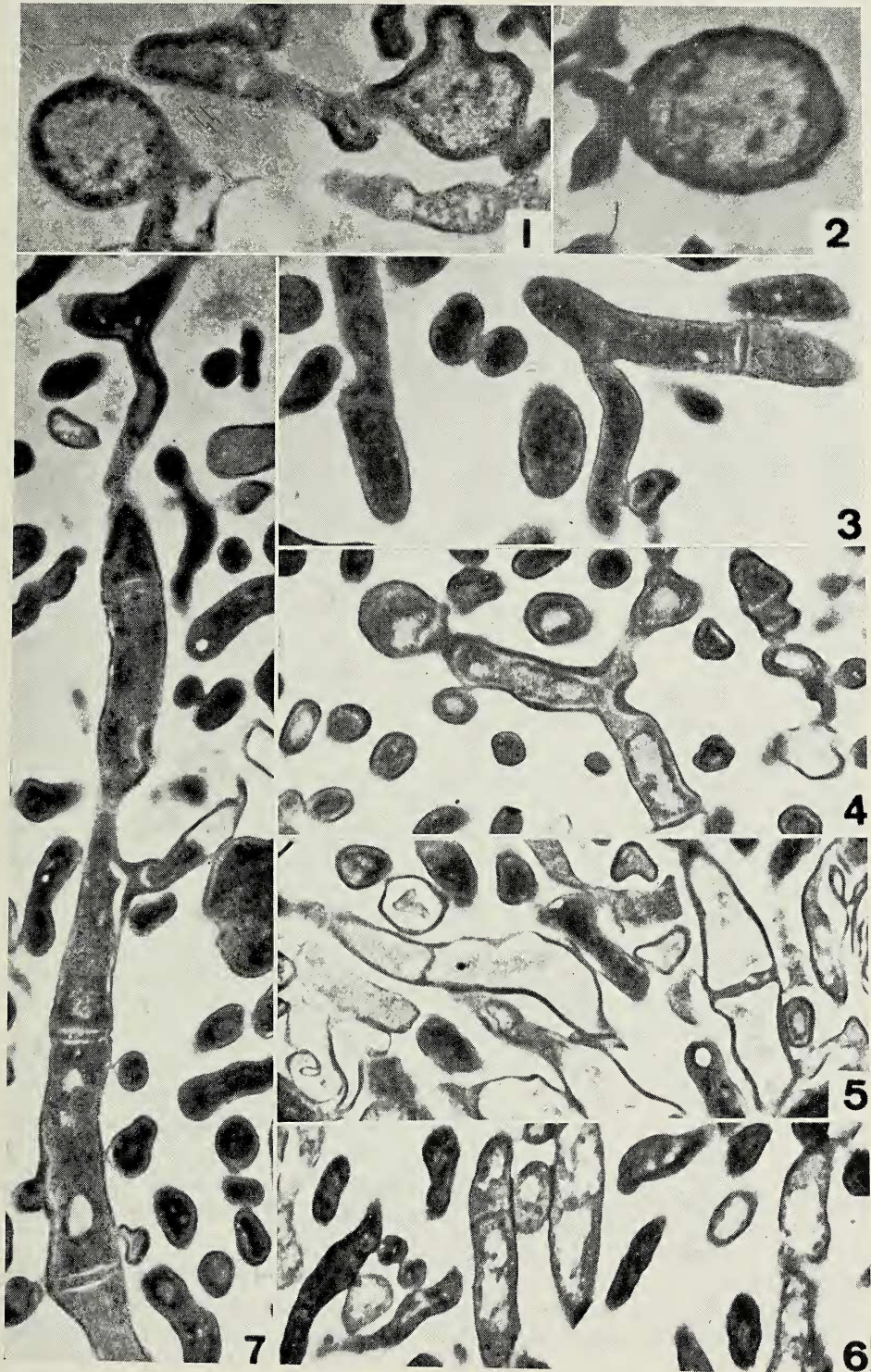
FIG. 3. *Micromonospora purpurea*, with septum and Y-shaped branching. $\times 25,000$.

FIG. 4. *Micromonospora purpurea*, with prespore in form of a constricted and bulbous hyphal tip; Y-shaped branching as in Figure 3. $\times 22,500$.

FIG. 5. *Micromonospora purpurea*, showing general cell morphology and septa of various thicknesses. $\times 22,500$.

FIG. 6. Example of septate hypha in pathogenic *Micromonospora* spp. $\times 22,500$.

FIG. 7. Longitudinal section through hypha of *Micromonospora purpurea*, demonstrating the frequent occurrence of septa. $\times 25,000$.



MATERIALS AND METHODS

Cultures were grown in broth on a Gyrotary shaker (New Brunswick Scientific Co.) at 200 rpm for three to four days. Sodium caseinate broth (Fred and Waksman, 1928) as modified by L. F. Potter (BBL No. 01-549) was the medium of choice. Fixation and embedding were performed according to the standard procedures of Kellenberger and Ryter (1958). Sections were cut on a Porter-Blum ultramicrotome and poststained with lead citrate. Electron micrographs were obtained on a Norelco EM 75 microscope.

RESULTS

The preliminary findings reported here demonstrate that the technique employed offers promise for its use in future investigations. All pictures taken clearly show the procaryotic nature of the three *Micromonospora* species: the nuclear areas are fibrillar and not surrounded by a membrane; the cytoplasm is densely granular and essentially devoid of lamellar organelles.

The hyphae of all three species sectioned (Figs. 1, 6, 7) show cross-walls or septa. These are particularly numerous in *M. purpurea* (Figs. 3, 4, 5, 7). Apparently there are no septal pores. Some branching was observed (Figs. 3, 4, 7). Spore formation seems to be initiated by the development of a heavy septum or plug within the hypha which isolates the apical end of the hypha as a roundish, somewhat enlarged structure filled with both cytoplasmic and nuclear material (Figs. 1, 4). The final spore (Fig. 2) has a sculptured wall. Wall sculpturing was shown by Leudemann and Brodsky (1964) on the spores of *M. echinospora*, but their pictures are completely devoid of inner detail.

DISCUSSION

Despite the preliminary nature of this report several points are worth emphasizing. The procaryotic nature of the cells has been established definitely for all three *Micromonospora* studied. Septa, which are particularly abundant in *M. purpurea*, are demonstrated clearly in all the strains considered: there should be no question of the occurrence of cross-walls in these three

micromonosporae. The interpretation of the branched cells is less clear. This might be interpreted as true branching or as the result of anastomosis of two hyphae. Leudemann and Brodsky (1965) reported sectoring in colonies of *M. carbonacea* which represented sporulating and non-sporulating areas. They compared this to the well-known heterocaryotic behavior in fungi. Both anastomosis and sectoring are suggestive evidence which calls for confirmation in the entire group of micromonosporae.

SUMMARY

Electron microscope studies of three species of the genus *Micromonospora* were made to clarify the scanty and conflicting information about cellular structure in this group. All materials revealed clearly the procaryotic nature of the cells and the presence of definite cross-walls in the hyphae.

REFERENCES

- AGRE, N. S. 1962. Electron-microscopic investigation of spores of *Micromonospora vulgaris*. *Microbiology USSR* (English trans.) 31:226-227.
- ARAI, T., Y. KOYAMA, S. KURODA, and H. HONDA. 1964. Biosynthesis of antibiotics by *Micromonospora*. *Kogyo Kagaku Zasshi* 67: 728-733.
- AVERY, R. J., and F. BLANK. 1951. On the chemical composition of the cell walls of the Actinomycetales and its relation to their systematic position. *Canadian J. Microbiol.* 1:140-143.
- CASTELLANI, A., M. M. X. DE BRITO, and M. R. PINTO. 1959. An actinomycete isolated from an autochthonous case of mycetoma in Portugal. *J. Trop. Med. Hyg.* 62:27-36.
- ERIKSON, D. 1941. Studies on some lake-mud strains of *Micromonospora*. *J. Bacteriol.* 41: 277-300.
- . 1947. Differentiation of the vegetative and sporogenous phases of the Actinomycetes. 1. The lipid nature of the outer wall of the aerial mycelium. *J. Gen. Microbiol.* 1:39-44.
- FRED, E. B., and S. A. WAKSMAN. 1928. Lab-

- oratory Manual of General Microbiology. McGraw-Hill Book Co., Inc., New York. 145 pp.
- JENSEN, H. L. 1930. The genus *Micromonospora* Ørskov, a little known group of soil micro-organisms. Proc. Linnean Soc. N. S. W. 55:231-248.
- 1932. Contributions to our knowledge of the Actinomycetales. III. Further observations on the genus *Micromonospora*. Ibid. 57:173-180.
- KELLENBERGER, E., and A. RYTER. 1958. Cell wall and cytoplasmic membrane of *Escherichia coli*. J. Biophys. and Biochem. Cytol. 4:323-325.
- KRASIL'NIKOV, N. A., and N. S. AGRE. 1965. The brown group of *Actinobifida chromogena* n. sp. Microbiology USSR (English trans.) 34:239-244.
- LEUEMANN, G. M., and B. C. BRODSKY. 1964. Taxonomy of gentamicin-producing *Micromonospora*. Antimicrobial Agents and Chemotherapy, 1963: 116-124. ASM, Ann Arbor.
- ——— 1965. *Micromonospora carbonacea* sp. n., an everninomycin-producing organism. Antimicrobial Agents and Chemotherapy, 1964: 47-52. ASM, Ann Arbor.
- ØRSKOV, J. 1923. Investigations into the Morphology of the Ray Fungi. Levin and Munksgaard, Copenhagen.
- POTTER, L. F., and G. E. BAKER. 1956. The microbiology of Flathead and Rogers lakes, Montana. I. Preliminary survey of microbial populations. Ecology 37:351-355.
- WAKSMAN, S. A. 1961. The Actinomycetes. Vol. II. The Williams and Wilkins Co., Baltimore. 363 pp.
- WEINSTEIN, M. J., G. M. LEUEMANN, E. M. ODEN, and G. H. WAGMAN. 1965. Everninomycin, a new antibiotic complex from *Micromonospora carbonacea*. Antimicrobial Agents and Chemotherapy, 1964:24-32. ASM, Ann Arbor.
- YAMAGUCHI, T. 1965. Comparison of the cell-wall composition of morphologically distinct actinomycetes. J. Bacteriol. 89:444-453.